Remarks

Accompanying this amendment is a petition and fee authorization for a three month extension of time.

The specification and claims have been reviewed in light of the office action to which this amendment is responsive. The claims have been amended to improve their form. The amendments do not add any new matter and are believed to place the application in condition for allowance.

The specification has been amended to delete sequences not set forth in the sequence listing. The deleted sequences are not essential for disclosure of the claimed invention, and deleting them obviates the need for a substitute sequence listing.

Turning now to the specific objections and rejections raised by the Examiner, the specification was objected to as failing to comply with the sequence rules. The amendments to the specification obviate this objection.

Claim 11 was objected to as informal. It has been cancelled.

Claims 10 and 11 were rejected for double patenting. Claims 10 and 11 have been cancelled.

Claims 8 and 11 were rejected under 35 USC 112, second paragraph.. Claim 8 has been amended to obviate this rejection and claim 11 has been cancelled.

Claim 9 was rejected under 35 USC 112, first paragraph. Claim 9 has been cancelled.

Claims 1-3 and 6-9 were rejected under 35 USC 112, first paragraph as broader than the enabling disclosure. Enablement for transformation of cells from rice and maize Type I and Type II callus and embryogenic cotton callus was acknowledged by the Examiner. The claims have been limited to transformation of hypocotyl-derived or cotyledon-derived cotton callus. Applicants reserve the right to pursue additional enabled subject matter in a divisional application.

Claims 1-3, 5, 8, and 9 were rejected under 35 USC 102(b) as anticipated by Frame et al. (Plant J., 1994, Vol. 6, pages 941-948). Frame et al. is directed to wiskers transformation of maize suspension cultures. All of the pending claims are directed to transformation of hypocotyl or cotyledon derived embryogenic cotton callus. Accordingly, the claimed subject matter is clearly not identically disclosed in the reference.

Claim 9 was rejected under 35 USC 102(b) as anticipated by, or obvious under 35 USC 103(a) over Kuehnle et al. Claim 9 has been cancelled.

Claims 1-9 were rejected under 35 USC 103(a) as unpatentable over Frame et al. in combination with Ishida et al. (Nature Biotech. 196, Vol. 14, pages 745-750) and Firoozabady et al. (Plant Mol. Biol., 1987, Vol. 10, pages 105-116). Ishida et al. was cited as disclosing Type I maize callus culture. Firoozabady et al. was cited as disclosing cotyledon-derived cotton callus cultures. The Examiner stated:

> It would have been obvious and within the scope of one of ordinary skill in the art at the time the invention was made to use the whisker-mediated transformation method of Frame et al. to transform other cell types, including the Type I callus of Ishida et al. or the cotyledon-derived cotton callus of Firoozabady et al. and regenerated transgenic plants therefrom. One would have been motivated to use the method with other plants and cell types, including callus cultures from cotton hypocotyl tissue, given the suggestion of Frame et al. that it can be used with other species for which regenerable cultures exist.

Applicants respectfully submit that the availability of regenerable cotton cultures would not have provided one of ordinary skill with reasonable confidence that whiskers could be used with such cultures to produce transgenic plants. The Examiner assumes that the only obstacle to extending use of whiskers to other species and other tissues is the availability of a regenerable culture. In the context of transformation of cotton, which has historically been known as a very difficult crop to transform, it was pointed out in US 5,004,863 that:

> The two processes, transformation and regeneration, must be complementary. It is possible to transform certain tissues or cell types which cannot be regenerated, and it is also possible to regenerate plant tissues of a number of different tissue and cell types which have not yet been successfully transformed.... The complementarity of the two processes must be such that the tissues which are successfully genetically transformed by the transformation process must be of a type and character, and must be in sufficient health, competency and vitality, so that the can be successfully regenerated into whole plants.

5,004,863 at column 3, lines 15-27.

The whiskers transformation process has a significant physical impact on the tissue being treated and has a negative impact on the vitality of cells. One of ordinary skill could not have been reasonably confident that whiskers transformation was complementary to hypocotyl or cotyledon derived cotton callus. Compared to maize suspension cultures or maize type II callus, hypocotyl or cotyledon derived cotton callus has a dense and organized structure. The cells are differentiated, and relative to maize suspension cultures, a relatively low number of the cells are meristematic. The mere fact that such

tissues are regenerable if they are not subjected to transformation, or that they are regenerable after transformation with agrobacterium would not lead one of ordinary skill to reasonably predict that such callus treated with wiskers would be regenerable.

Further, even if it were obvious to try whiskers transformation, the process involves a large number of parameters, including the relative amounts of fiber, DNA, and plant cells, oscillation method and time. Frame et al. stated:

> Our results suggest that whisker transformation of any species where regenerable suspension cultures exist should be possible once DNA delivery parameters have been established.

(Plant J., 1994, Vol. 6, at page 946).
Even that prediction, speculative as it was, was limited to suspension cultures, and recognized that delivery parameters would have to be established. If extended beyond suspension cultures, applicant believes that success could not reasonably have been predicted and would require undue experimentation.

Frame et al. also stated:

> Recently, we have obtained stably transformed lines from whisker treatment of embyrogenic callus derived from an elite stiff stalk maize inbred variety, indicating that the method can be extended to target tissues other than suspension cells.

(Plant J., 1994, Vol. 6, at page 946). To one of ordinary skill this statement would have raised more questions than it answered. Why was no data was presented? Why was the callus type not identified (was it type III? type II?)? Why were the process parameters not described? The statement would not have suggested to one familiar with the problems of transforming cotton that the whisker treatment disclosed for maize suspension cultures would work with cotton callus.

All of the teachings in the Frame et al. publication involve maize (not rice or cotton). Moreover, all of the methodologies and data pertain to suspension cell cultures (not callus). No guidance, beyond the mere mention that other tissues (beside suspension cultured cells) can work, is provided by Frame et al.

Undue experimentation would have been necessary and one of ordinary skill in the art would not have believed from the methods and data presented in Frame et al. that success was likely for anything beyond maize suspension cultures. Identifying the conditions necessary to deliver DNA into different types of cultures (beyond maize suspensions)

would have required significant experimentation. For example, the current application discloses a systematic set of parameter evaluations as they relate to DNA delivery via whiskers including genotype, tissue amount, DNA concentration, temperature pretreatment, osmotic treatment, whisker amount, whisker type, agitation vessel, and agitation device. Evaluating these, and perhaps other, variables for each target tissue would have been essential for success. What might work for maize suspension cultures may not work for cotton suspension cultures much less cotton or rice callus. Many variables would have needed to be manipulated and until a successful combination of parameters was identified, success could not have been predicted.

There are many types of embryogenic callus cultures each with its own morphology, friability, selectability, etc., even within a given species such as maize (Welter et al., 1995, Plant Cell Reports, Vol. 14, pages 725-729). One of ordinary skill would have exprected whisker transformation to differ across different types of embryogenic callus – especially callus from different species. Indeed, Type I rice callus is very different from embryogenic maize callus with respect to morphology, texture, growth, etc. and embryogenic cotton callus derived from hypocotyls or cotyledons is even more different. It would not have been obvious to one of ordinary skill in the art from Frame et al. that rice or cotton could be transformed with whiskers. The methods taught and data presented in Frame et al. would have been of little use for rice or cotton transformation.

Hypocotyl- and/or cotyledon-derived cotton callus has been classified into non-embryogenic and embryogenic depending on morphology and regenerative capacity (Shoemaker et al., 1986, Plant Cell Rep. 3:178-181). Non-embryogenic callus is comprised of a loose, friable mass of cells that do not exhibit a strong cytoplasm staining reaction and does not readily regenerate plants. Embryogenic callus appears as a tightly compact, densely cytoplasmic mass of cells capable of plant regeneration via somatic embryogenesis. Somatic embryos first appear as globular structures which gradually elongate and begin to show signs of cotyledonary development. Compared to maize and rice, embryogenic cotton callus is very hard and compact. No one would have predicted from Frame et al. or Firoozabady et al. that such tissue could be transformed with whiskers.

Ishida et al. and Firoozabady et al. do not teach whisker transformation nor mention how amenable their tissue cultures may be to such a method. DNA delivery via whiskers is a very different process than is transformation via Agrobacterium co-cultivation (physical vs. biological). There would have been no reason to suspect from Ishida et al. and/or Firoozabady et al. in combination with Frame et al. that rice or cotton could be transformed via whiskers. The methods taught and the data presented in Ishida et al. and Firoozababy et al. would have been of little use for whisker transformation.

Accordingly, the claimed invention is not obvious in view of the cited references. Reconsideration in light of the foregoing amendments and remarks is respectfully requested.

Respectfully submitted,

Donald R. Stuart

Donald R. Stuart
Registration No. 28,479
Phone: 317-337-4816

Dow AgroSciences LLC
Patent Section
9330 Zionsville Road
Indianapolis, Indiana 46268

Date: July 29, 2003